

Why Do Congo Red, Evans Blue, and Trypan Blue Differ in Their Complexation Properties?

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ABSTRACT: Congo Red, Evans Blue, and Trypan Blue dyes were evaluated in terms of their ability to form supramolecular systems in water solution. A geometric transformation set was defined to construct a supramolecular model system composed of eight dye molecules. The stability of the constructed multimolecular systems was estimated by molecular dynamics using AMBER 4.1 and DISCOVER force fields. The results essentially confirm the tendency toward self-assembly in each case. However, Congo Red and Evans Blue were found to form stable, continuous, ribbon-like supramolecular organizations, whereas Trypan Blue self-assembly appeared defective; some additional deviations from planarity seem to be the main reason for the disturbance in self-assembling. The extra rotation around the azo bonds in the Trypan Blue molecule is slightly extorted by the close proximity of sulfonic groups. This may also be the direct cause of the observed deviation from symmetry in the molecule of this dye. The results confirm that the self-assembling capability of the compounds studied correlates with the capacity to complex proteins, supporting the idea that supramolecularity may create specific ligation properties. © 2000 John Wiley & Sons, Inc. *J Comput Chem* 21: 656–667, 2000

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Introduction

Congo Red and some other related bis-azo dyes have recently become the focus of interest of many researchers due to the common use of these dyes in testing amyloid proteins, including Alzheimer plaque proteins and prions.¹ In recent years, however, many other nonamyloid proteins, basically those rich in β -structure, have also been found to bind these dyes when they become molten.² However, some destabilized, but yet not molten, proteins have also been observed to be capable of dye attachment.

Congo Red and many other bis-azo dyes have long been known to self-assemble in water solutions, but not until recently has this property been thought to be associated with ligation capability.^{3–10} It is of interest is that this capability concerns some proteins and that they may bind supramolecular fragments as single ligands.⁶ The mechanism of such binding is not yet clear, although the suggested adhesion of ribbon-like micellar fragments to peptide chains of β -conformation is increasingly convincing.

This study approaches the problem of properties that arise from the supramolecular organiza-

tion of Congo Red, Evans Blue, and Trypan Blue by comparing their properties through computer analysis (Fig. 1). The starting point is the significant difference observed in binding to proteins and self-assembly between the two isomeric dyes, Evans Blue and Trypan Blue.^{4,5,7} Studies of these two dyes offer a unique opportunity to approach the mechanism of dye-protein interaction by structural analysis of the ligand.

This work attempts to shed some light on the complexation mechanism of the studied dyes by using molecular dynamics simulation to compare the stability of their supramolecular organization. The lowest energy conformation and micellar organization formed by these azo dyes were searched using a minimization of the AMBER 4.1 potential energy function from a few starting conformations. The same starting conformations for Congo Red were taken to determine the optimal structure with the DISCOVER program of the INSIGHT II package.

Materials and Methods

PROGRAMS AND COMPUTERS

The molecular-mechanics calculations aimed at energy optimization of monomer and supramolec-

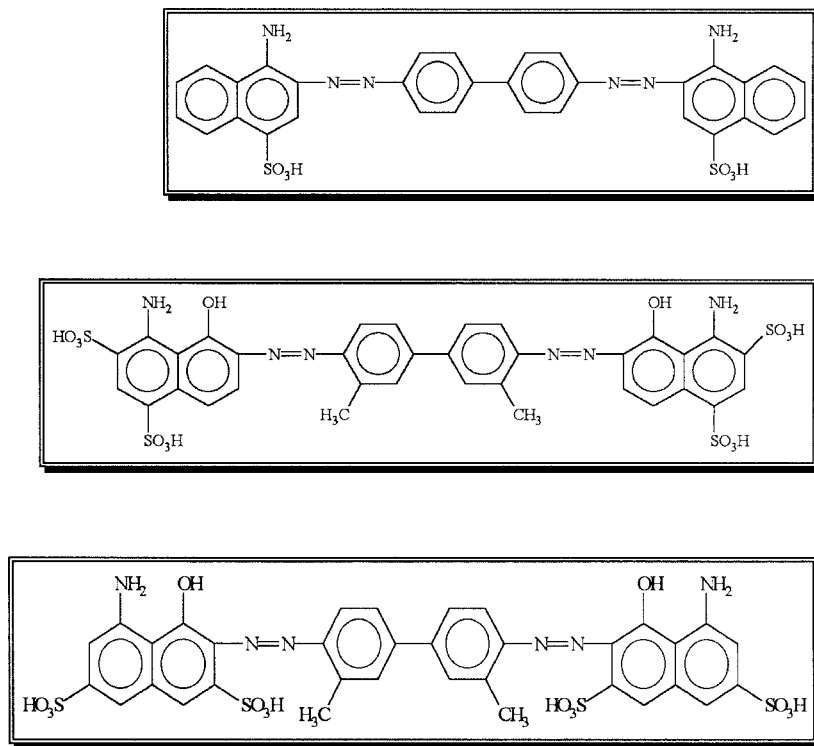


FIGURE 1. Chemical formulas of the dye molecules: Congo Red, Evans Blue, and Trypan Blue.

ular organization, and simulation of the molecular dynamics of the dyes, were done using AMBER 4.1¹¹ on an HP-Convex Exemplar SPP-1600/XA-32 computer. All calculations were done at the CYFRONET Academic Computer Center in Cracow.

The INSIGHT II calculations were performed using a Silicon Graphics Octane workstation.

SUPRAMOLECULAR STRUCTURE ORGANIZATION

The Resp method (as incorporated in AMBER 4.1) delivered the optimal structure and partial charge distributions of the monomers, which have been analyzed exhaustively.¹² The nonbonding parameters were taken from Woodcock et al.¹³

A set of geometric transformations was proposed earlier to define the mutual orientation of the monomer molecules in the supramolecular system.⁶ The geometry of the set of molecules is shown graphically in Figure 2. The source molecule, Congo Red *trans*, was placed with the central benzidine bond center of gravity in the center of the coordinate system, and its dipole moment vector was oriented along the Z-axis (along the X-axis for Congo Red *cis* and Evans Blue *trans*). The "image" molecules in the eight-molecule micellar system were created using three transformations: T_x (angstroms), translation along the X-axis; R_z (deg), rotation around the Z-axis; and T_z (angstroms), translation along the Z-axis. These transformations allow creation of a cylindrical system with helical symmetry. The set of

transformations provides a broad spectrum of different structures; for example, [$T_x = 0$, $R_z = 0$, $T_z \neq 0$] allows a column-like system to be created, where the molecules are superimposed in a parallel manner along the Z-axis. Helix-like columns can be created for the following set of parameters: [$T_x = 0$, $R_z > 0$, $T_z > 0$]. The set of parameters [$T_x > 0$, $R_z > 0$, $T_z > 0$] produces a cylinder-like organization of the molecules lying on its surface. The T_x parameter represents the radius of curvature of the helical system, R_z represents the angular displacement, and T_z determines the intermolecular distance dependent on the intermolecular interaction (i.e., the axial rise).

To avoid parallel mutual orientation of the dipole moments of the monomers, antiparallel orientation was introduced by rotating every second molecule in the octamer 180° around the long internal molecular axis.

ENERGY MINIMIZATION FOR SUPRAMOLECULAR SYSTEMS

The constructed octamers just presented were placed in a box filled with water (TIP3P)^{14,15} (minimum layer of water 3 Å). Energy minimization was a three-step procedure¹⁶: in the first step, the water molecule distribution was optimized in the presence of solute; in the second, the solute molecules were adapted to the surrounding water; and, in the third, the whole system was made flexible to fit the optimal mutual orientation. The procedure was performed for a dielectric constant of 1.0 and a cutoff distance of 12 Å.

MOLECULAR DYNAMICS SIMULATION

Molecular dynamics¹⁷ were simulated for liquid crystalline systems¹⁸ and the micellar systems.^{19,20}

The calculations were done using the leapfrog procedure²¹ with a timestep of 1 fs. The whole system was kept in a constant 300 K bath.²² All simulations represented 150-ps time periods without any intermolecular constraints. The bond lengths for all bonds were kept constant during the simulations, using the SHAKE procedure with 0.0005-Å tolerance.²³

The dielectric constant was 1.0 and the cutoff distance was 12 Å. Energy value and temperature were monitored every picosecond during the 150 ps of dynamics.

The simulation of molecular dynamics for the Congo Red supramolecular system using DISCOVER (cvff)²⁴⁻²⁶ was done under the same conditions as for the AMBER force field, in order to trace

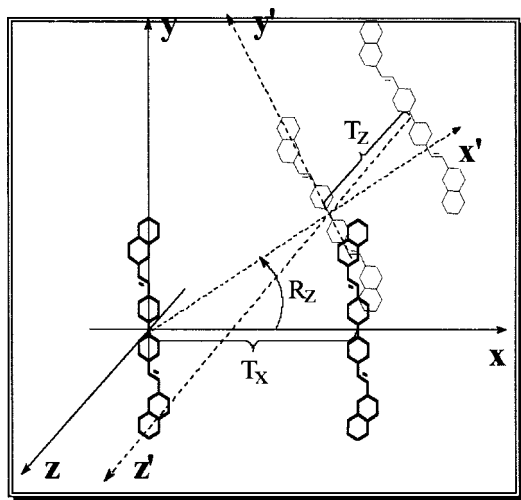


FIGURE 2. Graphic representation of T_x , R_z , and T_z geometric transformations used to construct supramolecular systems containing eight molecules of Congo Red, Evans Blue, and Trypan Blue.

the differences caused only by force-field parameterization.

ANALYSIS OF FINAL STRUCTURES

The structures that appeared during the last 25 ps of dynamics were taken for analysis. Structural analysis focused on the fluctuation of the Tx, Rz, and Tz values. Inter- and intramolecular interactions expressing the energetic stability of the system were evaluated using the ANAL module of the AMBER 4.1 program. The trajectories of each system were analyzed using the CARNAL module of AMBER 4.1.

Results and Discussion

MONOMER STRUCTURE OF CONGO RED, EVANS BLUE, AND TRYPAN BLUE

The optimal structures of Congo Red, Evans Blue, and Trypan Blue in monomer form were calculated using the Resp procedure as adopted in the AMBER 4.1 program.¹¹ Multiconformational one-degree fit was applied to the Congo Red molecule to unify the partial charge distributions for the trans and cis isomers. Two-degree fit was applied to the Evans Blue molecule. The Trypan Blue trans conformation partial charge distribution was found after single-conformational two-degree fitting. The optimal geometry and partial charge distribution is presented in Table I of the Supplementary Material.

The structures of all dyes were found to be nonplanar, with the dihedral angle between two benzene rings in the 40° to 45° range. The nonplanarity of Trypan Blue was additionally caused by the di-azo-bond dihedral angles, which were found to differ versus 180°.

The presence of polar groups was the main reason for the high dipole moment. In the Congo Red trans form, the dipole moment was found to be directed perpendicularly to the averaged molecular plane, whereas for the cis form it was directed parallel. The dipole moment for the Evans Blue trans form was calculated to be parallel to the averaged molecular plane, and perpendicular in the cis form. The dipole moment of Trypan Blue was very sensitive to small changes in molecular structure, especially due to the nonplanarity of the symmetrical part of the molecule; consequently, this parameter could not be considered a structural attribute.

The general characterization concerns the planarity of the molecules, which appeared different in Trypan Blue. The only torsion in Congo Red and Evans Blue was present on the benzidine bond. Additional nonplanarity appeared in Trypan Blue on the di-azo bonds, caused by the strong 1-4 interaction between nitrogen (di-azo bond) and sulfur atoms.

OCTAMER STRUCTURES OF CONGO RED, EVANS BLUE, AND TRYPAN BLUE

Eight different mutual orientations of Congo Red, four of Evans Blue, and two of Trypan Blue molecules were selected to emphasize their influence on intermolecular interaction. The following notation was used to distinguish the different mutual orientations of the molecules in the supramolecular system:

1. *T* and *C*—trans and cis, respectively.
2. Direct (parallel), $\Rightarrow\Rightarrow$, $\uparrow\uparrow$ and reversed (antiparallel) $\Rightarrow\Leftarrow$, $\uparrow\downarrow$ orientations of the dipoles.
3. The values in parentheses express the geometric parameters Tx, Rz, and Tz.

Thus, the following forms were analyzed in this work:

<i>T</i> _{CR} (0, -13, 4) ($\uparrow\downarrow$)	<i>T</i> _{EB} (0, -10, 4) ($\uparrow\downarrow$)
<i>C</i> _{CR} (0, -13, 4) ($\Rightarrow\Leftarrow$)	<i>C</i> _{EB} (0, -10, 4) ($\Rightarrow\Leftarrow$)
<i>T</i> _{CR} (0, -13, 4) ($\uparrow\uparrow$)	<i>T</i> _{EB} (0, -10, 4) ($\uparrow\uparrow$)
<i>C</i> _{CR} (0, -13, 4) ($\Rightarrow\Rightarrow$)	<i>C</i> _{EB} (0, -10, 4) ($\Rightarrow\Rightarrow$)
<i>T</i> _{CR} (18.6, 9, 4) ($\uparrow\downarrow$)	
<i>C</i> _{CR} (18.6, 9, 4) ($\Rightarrow\Leftarrow$)	
<i>T</i> _{CR} (0, 0, 4) ($\uparrow\downarrow$)	<i>T</i> _{TB} (0, -10, 4) ($\uparrow\downarrow$)
<i>C</i> _{CR} (0, 0, 4) ($\Rightarrow\Leftarrow$)	<i>T</i> _{TB} (0, -10, 4) ($\uparrow\uparrow$)

Only the forms shown in bold were selected to be presented in detail in this article. Forms shown in italics were taken for INSIGHT II calculations.

Satisfactory energy stabilization for the octamer Congo Red system was reached for all forms studied during the 150-ps molecular dynamics (Fig. 3 of Supplementary Material).

To evaluate the stability of the system in terms of geometric stabilization, the transformation parameters were traced for the entire duration of the dynamics simulation (Fig. 4 of Supplementary Material). The averaged 25 structures created during the last 25 ps of dynamics were considered to be the final structure of the micellar system (Fig. 5). The intermolecular energy components and the stability

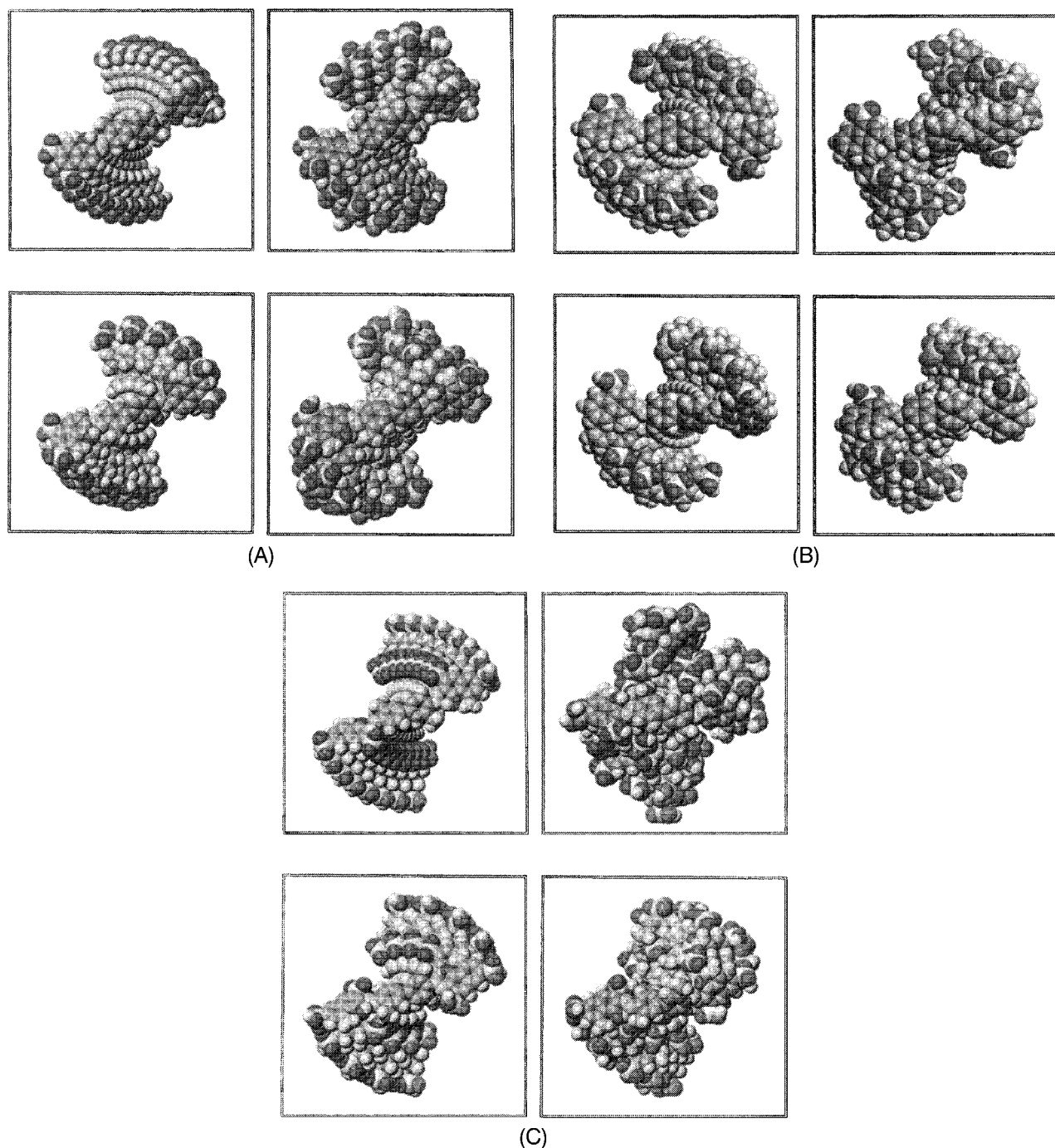


FIGURE 5. Starting (left column) and final (right column) (after 150 ps of molecular dynamics simulation) structure of supramolecular systems of: (A) Congo Red. $T_{CR}(0, -13, 4) \downarrow \uparrow$ (upper row) and $C_{CR}(0, -13, 4) \Rightarrow \Leftarrow$ (lower row). (B) Evans Blue. $T_{EB}(0, -13, 4) \downarrow \uparrow$ (upper row) and $T_{EB}(0, -13, 4) \Rightarrow \Leftarrow$ (lower row). (C) Trypan Blue. $T_{TB}(0, -10, 4) \downarrow \uparrow$ (upper row) and $T_{TB}(0, -10, 4) \uparrow \uparrow$ (lower row).

of the geometric parameters were measured to determine the stability of the supramolecular organization (see Tables II, IV, and VI in this article and Tables III and V of the Supplementary Material).

The stability of two Congo Red micellar organizations was also determined using the DISCOVER force field (cvff): $T_{CR}(0, -13, 4) \uparrow \downarrow$ and $T_{CR}(18.6, 9, 4) \uparrow \downarrow$. The energy characteristics of

TABLE II. Energetic and Geometric Characteristics of Congo Red in the $T_{CR}(0, -13, 4) \uparrow\downarrow$ and $C_{CR}(0, -13, 4) \Rightarrow\Leftarrow$ Forms of Octamer.

Octamer	$T_{CR}(0, -13, 4) \uparrow\downarrow$				$C_{CR}(0, -13, 4) \Rightarrow\Leftarrow$			
	Internal	vdW	Electr	Total	Internal	vdW	Electr	Total
CR1	69.008	129.683	-79.814	18.876	69.450	31.470	-80.374	20.546
CR1 – CR2	—	-44.522	-7.249	-51.771	—	-43.630	-9.038	-52.668
CR2	63.196	31.865	-80.861	14.201	67.659	31.459	-78.932	20.187
CR2 – CR3	—	-42.189	-4.058	-46.247	—	-44.449	0.369	-44.080
CR3	69.769	31.715	-80.437	21.047	67.714	31.245	-81.803	17.156
CR3 – CR4	—	-44.126	-8.062	-52.189	—	-44.718	-11.675	-56.393
CR4	68.741	31.647	-79.512	20.877	67.443	32.172	-79.595	20.020
CR4 – CR5	—	-43.057	-4.962	-48.020	—	-43.518	-2.737	-46.254
CR5	68.829	31.711	-78.723	21.817	66.937	31.737	-79.192	19.483
CR5 – CR6	—	-45.221	-7.550	-52.770	—	-43.396	-9.714	-53.109
CR6	66.583	29.832	-79.997	16.418	64.086	31.885	-80.635	15.336
CR6 – CR7	—	-43.588	-3.696	-47.285	—	-44.412	-0.835	-45.247
CR7	66.674	31.230	-78.619	19.285	67.173	31.738	-79.408	19.503
CR7 – CR8	—	-42.700	-7.763	-50.463	—	-44.694	-9.735	-54.429
CR8	66.674	29.328	-79.632	20.467	67.173	30.960	-78.295	22.818
Total internal	543.570	247.010	-637.593	152.988	540.615	252.667	-638.233	155.049
Total inter	—	-328.453	-39.105	-367.557	—	-333.147	-37.297	-370.444
Total total	543.570	-81.442	-676.696	-214.569	540.615	-80.479	-675.53	-215.394
	<i>-7.691</i>	<i>-604.581</i>	<i>-128.621</i>	<i>-516.678</i>	<i>-12.492</i>	<i>-364.881</i>	<i>-184.943</i>	<i>-430.093</i>

Octamer	$T_{CR}(0, -13, 4) \uparrow\downarrow$			Octamer transform	$C_{CR}(0, -13, 4) \Rightarrow\Leftarrow$			Octamer
	Tx (Å)	Rz (deg)	Tz (Å)		Tz (Å)	Rz (deg)	Tx (Å)	
CR1	1.105	-5.243	3.512	CR1 – CR2	3.514	-12.125	0.406	CR1
CR2	0.395	-20.470	3.552	CR2 – CR3	3.493	-8.871	0.474	CR2
CR3	2.101	-3.027	3.477	CR3 – CR4	3.350	-11.195	0.637	CR3
CR4	0.908	-18.099	3.311	CR4 – CR5	3.337	-10.114	0.699	CR4
CR5	1.328	-5.346	3.437	CR5 – CR6	3.687	-15.371	1.044	CR5
CR6	1.587	-19.660	3.491	CR6 – CR7	3.206	-14.950	1.336	CR6
CR7	0.314	-4.863	3.642	CR7 – CR8	3.502	-13.918	0.271	CR7
CR8	1.488						0.401	CR8
	1.153	10.958	3.489	Mean	3.441	12.361	0.658	
	<i>1.003</i>	<i>10.736</i>	<i>4.042</i>		<i>3.983</i>	<i>8.231</i>	<i>0.582</i>	

these two forms and the estimates of the geometric parameters’ stability in these calculations agreed with those obtained using AMBER 4.1. The estimate of intermolecular interaction energy, calculated using the DISCOVER (cvff) force field, provides confirmation that the supramolecular system of Congo Red was stable (Table II of this article—data given in italics).

SHARED CHARACTERISTICS OF CONGO RED, EVANS BLUE, AND TRYPAN BLUE SUPRAMOLECULAR ORGANIZATIONS

The calculations for two forms of the supramolecular organization of Congo Red, $T_{CR}(0, -13, 4) \uparrow\uparrow$ and $T_{CR}(0, -13, 4) \uparrow\downarrow$, showed the smallest difference in the distributions of intermolecular inter-

TABLE IV. Energetic and Geometric Characteristics of Evans Blue in the $T_{EB}(0, -10, 4) \uparrow\uparrow$ and $T_{EB}(0, -10, 4) \uparrow\downarrow$ Forms of Octamer.

Octamer	$T_{EB}(0, -10, 4) \uparrow\uparrow$				$T_{EB}(0, -10, 4) \uparrow\downarrow$			
	vdW	Electr	Hbond	Total	vdW	Electr	Hbond	Total
EB1	40.407	-115.679	-0.226	14.429	37.973	-113.806	-0.368	15.150
EB1 – EB2	-52.445	2.898	-0.207	-49.755	-56.357	1.585	-0.006	-54.778
EB2	37.277	-113.043	-0.493	11.023	38.455	-115.460	-0.337	11.490
EB2 – EB3	-52.243	-0.290	-0.017	-52.55	-53.077	1.505	-0.005	-51.577
EB3	41.052	-115.703	-0.537	14.358	40.938	-120.687	-0.336	6.474
EB3 – EB4	-53.112	-3.134	-0.176	-56.422	-58.394	-3.662	-0.010	-62.066
EB4	39.289	-113.993	-0.552	12.246	38.211	-114.628	-0.361	9.520
EB4 – EB5	-51.135	0.147	-0.003	-50.990	-55.330	0.989	-0.030	-54.371
EB5	38.268	-116.730	-0.298	6.570	38.580	-114.968	-0.459	8.460
EB5 – EB6	-53.746	-6.146	-0.227	-60.119	-57.320	5.390	-0.006	-51.935
EB6	38.998	-114.369	-0.455	7.751	39.365	-117.802	-0.308	7.143
EB6 – EB7	-52.351	2.824	-0.800	-49.607	-55.786	8.968	-0.023	-46.842
EB7	39.363	-119.433	-0.487	11.508	38.419	-110.898	-0.302	13.374
EB7 – EB8	-53.646	-1.366	-0.147	-55.160	-57.541	-4.806	-0.007	-62.354
EB8	37.647	-113.903	-0.375	12.814	36.470	-114.416	-0.778	8.025
Total intra-	312.321	-922.852	-3.425	90.698	308.410	-922.665	-3.252	79.636
Total inter-	-393.677	1.307	-0.857	-393.227	-423.943	18.085	-0.087	-405.945
Total total	-81.378	-921.546	-4.281	-302.528	-115.532	-904.580	-3.338	-326.309

Octamer	$T_{EB}(0, -10, 4) \uparrow\uparrow$			Octamer transform	$T_{EB}(0, -10, 4) \uparrow\downarrow$			Octamer
	Tx (Å)	Rz (deg)	Tz (Å)		Tz (Å)	Rz (deg)	Tx (Å)	
EB1	0.985	-10.773	3.313	EB1 – EB2	3.493	-14.279	0.498	EB1
EB2	1.196	-15.419	3.274	EB2 – EB3	3.400	-16.858	1.597	EB2
EB3	1.508	-19.289	3.451	EB3 – EB4	3.432	-9.494	1.199	EB3
EB4	2.330	-7.466	3.308	EB4 – EB5	3.444	-10.246	0.732	EB4
EB5	1.926	-16.960	3.439	EB5 – EB6	3.442	-8.012	1.250	EB5
EB6	0.778	-12.071	3.404	EB6 – EB7	3.424	-10.152	0.732	EB6
EB7	0.488	-7.799	3.502	EB7 – EB8	3.531	-9.398	0.595	EB7
EB8	0.712						1.052	EB8
	1.240	-12.825	3.384	Mean	3.463	-11.115	0.976	

action energy and geometric stabilization (Figs. 6 and 7). Comparison of the self-assembly tendencies of all three dyes suggests that Congo Red represented the most ordered system.

Trypan Blue, $T_{TB}(0, -10, 4) \uparrow\downarrow$, represented the most energetically and geometrically unstable multimolecular system organization, exceptional among the three dyes. Supramolecular instability can be seen particularly for the fourth and fifth molecules in the micelle, expressed as a large de-

viation of energy values and geometric parameters with respect to other neighboring molecules (Figs. 6 and 7). Trypan Blue has very low internal energy, so its supramolecular system is dominated by its internal interaction rather than elastic adaptation for intermolecular interaction. The nonplanarity on the di-azo bonds in the Trypan Blue molecules disturbs the formation of a long, continuous molecular organization, and it makes the formation of such an organization improbable (Fig. 7).

TABLE VI. Energetic and Geometric Characteristics of Trypan Blue in $T_{TB}(0, -10, 4) \uparrow\uparrow$ and $T_{TB}(0, -10, 4) \uparrow\downarrow$ Forms of Octamers.

Octamer	$T_{TB}(0, -10, 4) \uparrow\uparrow$				$T_{TB}(0, -10, 4) \uparrow\downarrow$			
	vdW	Electr	Hbond	Total	vdW	Electr	Hbond	Total
TB1	31.350	-157.175	-0.826	-43.382	30.622	-152.362	-0.883	-38.627
TB1 – TB2	-55.349	-8.297	-0.159	-63.805	-55.761	-5.196	-0.104	-61.061
TB2	32.622	-156.363	-0.908	-39.573	31.320	-153.756	-0.825	-39.523
TB2 – TB3	-52.010	-2.484	-0.067	-54.561	-56.497	-16.505	-0.010	-73.012
TB3	31.428	-158.583	-0.823	-44.250	31.883	-158.428	-0.804	-43.669
TB3 – TB4	-56.270	-2.819	-0.329	-59.417	-53.627	-4.743	-0.044	-58.414
TB4	31.535	-155.656	-0.868	-40.769	31.241	-156.494	-0.776	-40.669
TB4 – TB5	-41.976	9.074	-0.041	-32.942	-55.907	-3.647	-0.121	-59.676
TB5	32.579	-154.506	-0.762	-34.324	30.496	-155.993	-0.865	-44.329
TB5 – TB6	-54.208	-1.450	-0.149	-55.806	-56.263	-2.938	-0.063	-59.264
TB6	31.371	-157.794	-0.803	-39.662	31.738	-153.854	-0.777	-38.992
TB6 – TB7	-57.723	-9.308	-0.174	-67.205	-56.348	1.136	-0.284	-55.495
TB7	32.295	-158.720	-0.814	-40.476	31.394	-160.705	-0.808	-45.536
TB7 – TB8	-55.847	-10.993	-0.175	-67.015	-55.167	-11.554	-0.005	-66.725
TB8	29.840	-151.828	-0.831	-35.812	30.643	-153.692	-0.809	-38.337
Total intra-	253.019	-1250.625	-6.635	-318.246	249.337	-1245.283	-6.548	-329.679
Total inter-	-400.340	-23.960	-1.093	-425.393	-418.465	-46.534	-0.630	-465.628
Total total	-147.321	-1274.585	-7.729	-743.640	-169.127	-1291.817	-7.178	-795.310

Octamer	$T_{TB}(0, -10, 4) \uparrow\uparrow$			Octamer	$T_{TB}(0, -10, 4) \uparrow\downarrow$			Octamer
	Tx (Å)	Rz (deg)	Tz (Å)		Tz (Å)	Rz (deg)	Tx (Å)	
TB1	3.307	-6.137	3.170	TB1 – TB2	3.567	-5.525	0.446	TB1
TB2	2.431	-6.698	3.925	TB2 – TB3	3.430	-16.251	0.341	TB2
TB3	4.399	-17.396	3.464	TB3 – TB4	3.436	-9.400	0.335	TB3
TB4	3.873	-33.871	3.359	TB4 – TB5	3.563	-21.773	0.471	TB4
TB5	2.521	-11.726	3.718	TB5 – TB6	3.476	-4.742	0.727	TB5
TB6	2.642	-14.508	3.329	TB6 – TB7	3.527	-24.101	0.415	TB6
TB7	3.407	-14.366	3.590	TB7 – TB8	3.533	-4.231	0.289	TB7
TB8	3.180						0.650	TB8
	3.220	-14.958	3.508	Mean	3.505	-12.289	0.459	

Conclusions

Congo Red and two related isomeric dyes, Evans Blue and Trypan Blue, were selected for studies to determine why they differ in their properties, including self-assembly and interaction with proteins. It is increasingly evident that Congo Red and other similar dyes that form chromonic, ribbon-like micellar mesophases in water solutions may

interact with proteins as single ligands even while preserving their supramolecular character in complexation.^{4, 6, 7, 27, 28} The unusual Congo Red property concerning the possible single molecular²⁹ and supramolecular complexation suggests that this dye is amenable to attachment to different polymeric conformations, even including occlusion at protein interfaces.³⁰ However, its binding to β -peptide chains seems favored.^{2, 4, 6-8, 31, 32} Two isomeric dyes, Evans Blue and Trypan Blue, whose

reactivities as protein ligands differ dramatically, were chosen for structural computational studies to assess the origin of such differences.

The evidence for the ribbon-like geometry of the Congo Red organization considered as starting structures in this work came from previous experimental studies aimed at identifying (by NMR analysis) the particular Congo Red proton most significantly affected by the sulfonic group, which comes close to it in the transition to micellar organization.²⁷ This information, together with the independent finding that the central hydrophobic fragment of the dye is critical for stacking of the molecules in self-assembling, allowed the micelle organization to be defined. Microcalorimetric analysis has convincingly indicated that efficient shielding of charged groups at high ionic strength is necessary to prevent repulsion and allows close stacking of Congo Red molecules.²⁷ The charged molecules (water, low salt concentration) failed to form a supramolecular organization, so the no-charge Congo Red molecule

was used for simulation to approach the actual micellization process.

The structural similarity of the studied dyes was verified experimentally by comparing the stability of mixed micellar organizations.⁶ It was found that, although Evans Blue dye molecules form relatively stable mixed micellar derivatives with Congo Red, and while both may interact with proteins as supramolecular ligands, Trypan Blue is poorly engaged in self-assembling and this type of complexation.^{5,6}

These studies connect the observed differences regarding Trypan Blue with the specific location of the sulfonic groups in that molecule. The localization of the charged sulfonic groups in the central fragment of the dye molecule and, in particular, their close proximity to the di-azo bonds, causes the extra polarization effects and exerts some deviation in planarity. It significantly decreases the probability of the dye to form a stable supramolecular organization.

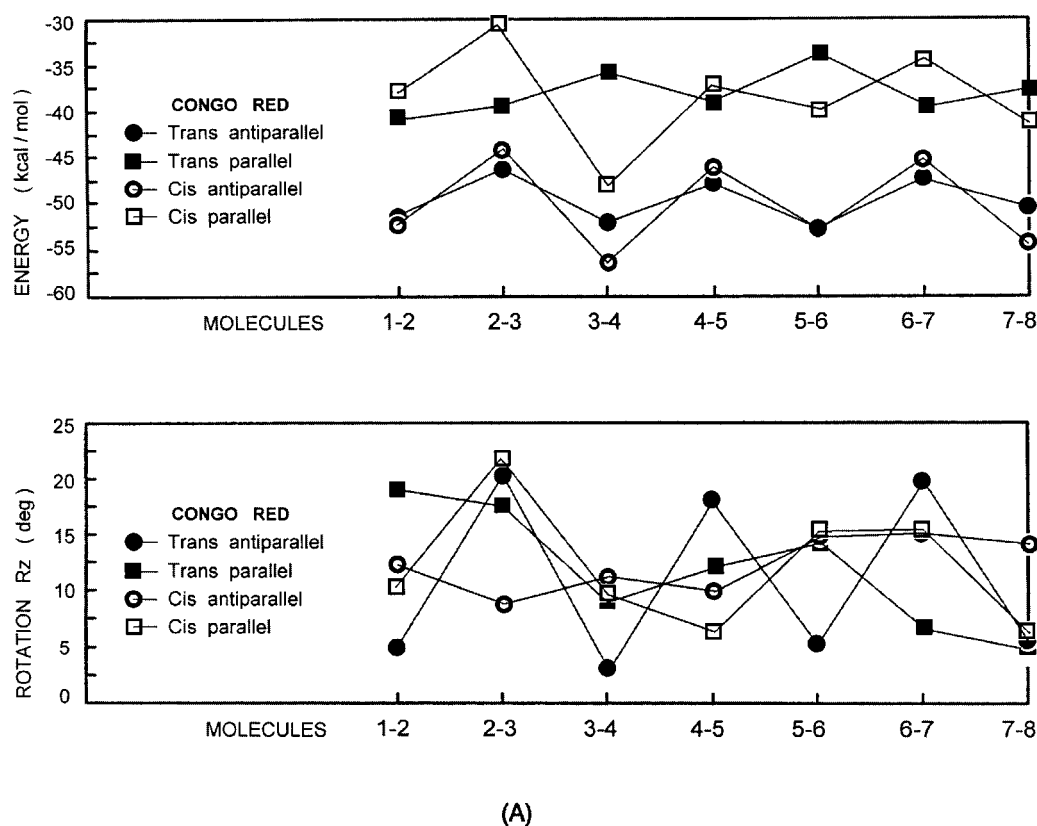
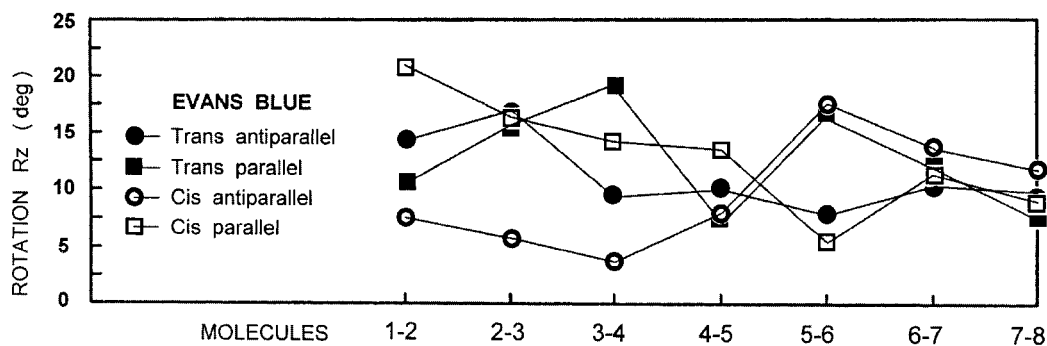
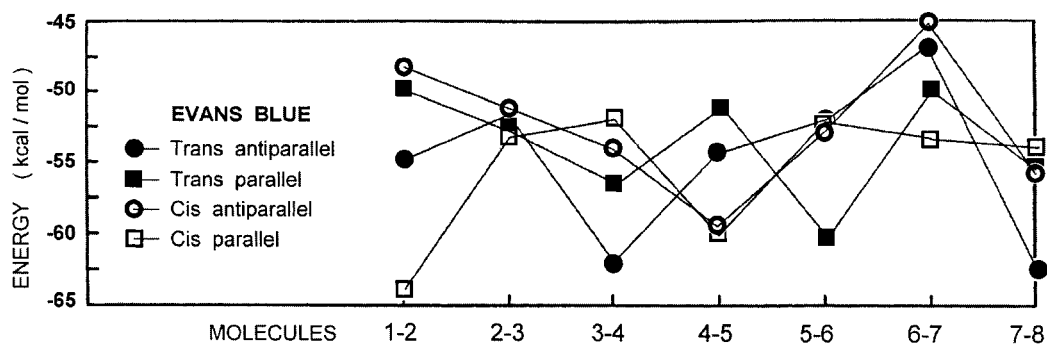
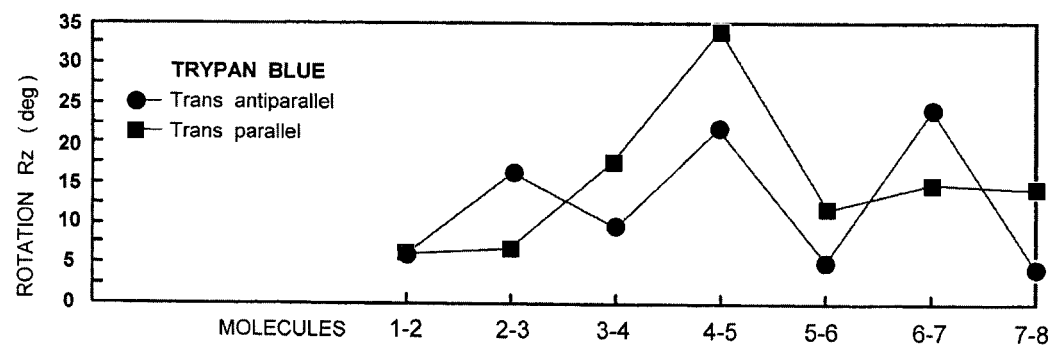
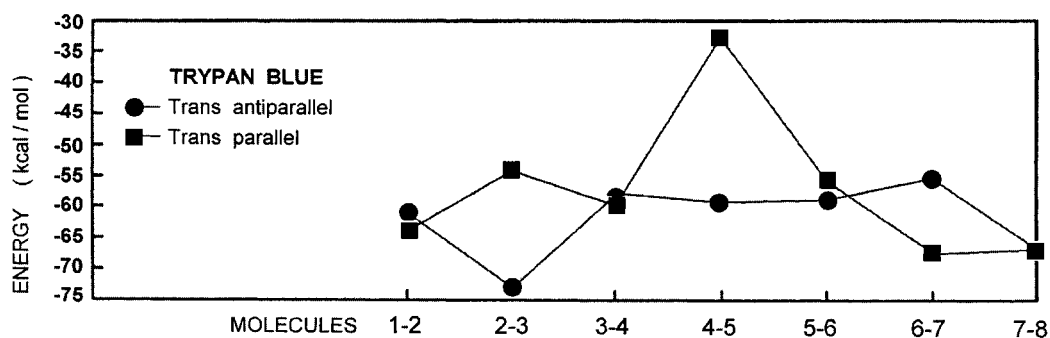


FIGURE 6. Supramolecular organization stability of intermolecular interaction and geometric parameter R_z (angstroms) in: (A) Congo Red. (Filled circles) $T_{CR}(0, -10, 4) \downarrow \uparrow$; (filled squares) $T_{CR}(0, -10, 4) \uparrow \uparrow$; (open circles) $C_{CR}(0, -10, 4) \Rightarrow \Leftarrow$; (open squares) $C_{CR}(0, -10, 4) \Rightarrow \Rightarrow$. (B) Evans Blue. (Filled circles) $T_{EB}(0, -10, 4) \downarrow \uparrow$; (filled squares) $T_{EB}(0, -10, 4) \uparrow \uparrow$; (open circles) $C_{EB}(0, -10, 4) \Rightarrow \Leftarrow$; (open squares) $C_{EB}(0, -10, 4) \Rightarrow \Rightarrow$. (C) Trypan Blue. (Filled circles) $T_{TB}(0, -10, 4) \downarrow \uparrow$; (filled squares) $T_{TB}(0, -10, 4) \uparrow \uparrow$.



(B)



(C)

FIGURE 6. (Continued)

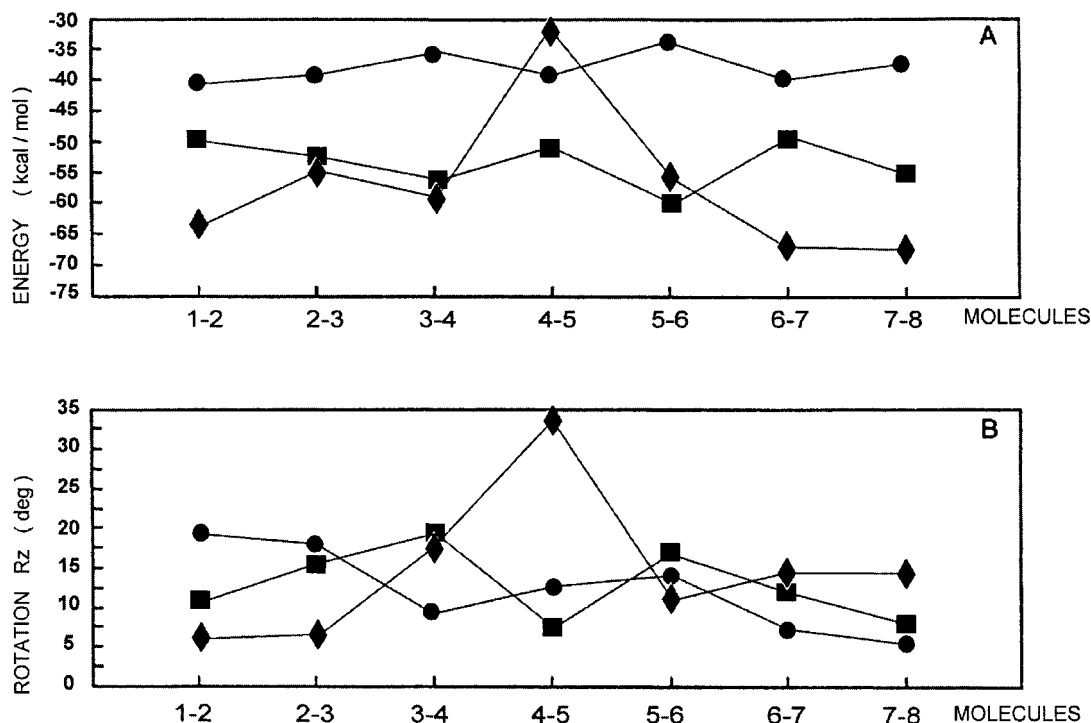


FIGURE 7. Supramolecular organization of (circles) Congo Red, (squares) Evans Blue, and (diamonds) Trypan Blue expressed by: (A) intermolecular interaction [$T(0, -10, 4) \uparrow\uparrow$] and [$T(0, -10, 4) \downarrow\downarrow$]; and (B) rotational R_z parameter [$T(0, -10, 4) \uparrow\uparrow$, $T(0, -10, 4) \downarrow\downarrow$].

The findings explain the observed differences in the complexation properties of the dyes and support an earlier suggestion that supramolecularity may create new ligation properties.⁷ The periodic structure of the polypeptide chains of the β -conformation corresponds to ribbon-like dye micelles, with their ordered arrangements and largely exposed hydrophobic portion.⁶

The calculations in this work were done with two widely used programs designed for molecular modeling. Particularly suggestive is the similarity of the data obtained for supramolecular systems using the DISCOVER and AMBER force fields—programs with obvious differences in parameterization. This argues in favor of the authenticity of the data.

Although the mechanism of dye-protein complexation is still not very clear, it is increasingly clear that tightly associated ribbon-like micellar supramolecular ligands have particular properties for binding proteins.

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